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ABSTRACT

These guides are four of several prepared through the F.D.A.'s Science Project Series for senior high school chemistry students and teachers investigating the quality of constituents of foods through experimentation. Each eight page pamphlet gives background information on the subject, equipment and reagents needed for the experiment, the technique, procedure, and special cautions to follow, time required for the project, questions for discussion, and a bibliography of related information. (BL)

ED045394

Identity of artificial color on oranges

FDA's SCIENCE PROJECT SERIES

U.S. Department of Health, Education, and Welfare
Public Health Service
Consumer Protection and Environmental Health Service
Food and Drug Administration

U.S. DEPARTMENT OF HEALTH, EDUCATION & WELFARE
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SE 010 109

Adapted from *Journal of the Association of Official Agricultural Chemists* XLIII, No. 2 (1960), 274.

Level Senior high school chemistry.

Technique Paper chromatography.

Time required Two hours.

SPECIAL CAUTIONS Preparation of ether-mineral oil solution for treatment of the chromatogram papers should be carried out in the hood.

Evaporation of chloroform solutions should also be carried out in the hood, over a steam bath if available.

IDENTITY OF ARTIFICIAL COLOR ON ORANGES

presented by the
Educational Services Staff
Food and Drug Administration

Sometimes oranges get ripe as far as taste is concerned before their skins turn completely orange. The development of the orange color in the peel is sometimes hastened artificially by exposing the oranges to ethylene gas in a special chamber designed for the purpose. The ethylene gas reacts with the pigmenting material in the peel to develop the color very much as it would naturally develop if the orange were left on the tree longer. Nothing is added to these oranges, and no special labeling is required.

But not all oranges respond satisfactorily to the ethylene treatment, and growers prefer to use artificial color. You may have noticed that some oranges are individually stamped "Color Added" or "Artificially Colored," while others do not bear this stamp. The uncolored oranges may be streaked with green, although they are ripe to the taste.

The color is permitted for use in making the oranges more attractive provided they are in fact ripe and otherwise of acceptable quality, and provided the consumer is notified by the stamp that artificial color has been used. The Federal Food, Drug, and Cosmetic Act (FD&C Act) prohibits the use of the color, however, when it would serve to conceal inferiority or damage, or to make the product appear better than it really is.

The color most often used on oranges is called Citrus Red No. 2. It was developed specifically for the purpose after it was shown that a previously permitted orange color could produce injury to test animals when it was consumed in large quantities.

Like all other colors used in foods, drugs, and cosmetics, the colors used on oranges must be specifically authorized for this use, and the amount used must be within the safe tolerance limit set by the Food and Drug Administration. In addition, a sample from every batch of the color produced by the manufacturer must be submitted to the Food and Drug Administration for tests. If the color is found to be pure and suitable for use, it is "Certified."

The color previously permitted on oranges, but now banned as not having been proved safe, is called Oil Red XO. It is possible—although unlikely—that some growers or shippers might use this nonpermitted color by mistake.

Perhaps it is more likely that Citrus Red No. 2 would be used, but the oranges not stamped. Such undeclared use of color would be a violation of the Federal Food, Drug, and Cosmetic Act and of State laws.

The *spectrophotometric absorption curve* of a color is preferred as a means of identification. However, in the absence of a suitable spectrophotometer, the probable identity of a color can be determined by other means, such as chromatographic properties.

In the following experiment, you can use a paper chromatographic technique to test colored oranges to determine that the proper color has been used, and to determine whether undeclared color was used on oranges not stamped "Color Added."

Experiment

Problem

To determine by ascending paper chromatography which colors are added to artificially-colored oranges, and whether colors have been used on oranges without proper labeling.

Equipment needed:

2 funnels, 125 mm.
6 glass rods
2 pipettes, 25 ml.
2 Erlenmeyer flasks, 125 ml.
2 beakers, 100 ml.
1 micro pipet; a capillary or melting point tube drawn to a fine point
1 steam bath
1 funnel support
2 sheets Whatman No. 3 MM filter paper, 7″ x 9″
1 chromatographic tank, inside dimensions 8″ x 9″ x 4″. Any glass or stainless steel tank of the approximate size will do. Small aquariums are useful and inexpensive.

Reagents:

cotton
oranges, at least 3 stamped "Color Added" and 3 not so stamped
chloroform, 300 ml.
light mineral oil, 5 grams
ethyl ether, 95 ml.
acetone, 200 ml.
distilled or deionized water, 100 ml.
*Citrus Red No. 2, 1-(2, 5-dimethoxphenylazo)-2 naphthol, about 0.1 gram
*Oil Red XO, 1-xylylazo-2-naphthol, about 0.1 gram

Teachers may obtain small quantities of Citrus Red No. 2 and Oil Red XO free of charge by writing on an official letterhead to:

Mr. Raymond V. Leary, Product Manager
National Aniline Division, Allied Chemical Corporation
40 Rector Street, New York, N. Y. 10006

Advance Preparation

Place the Whatman filter paper sheet so that the 9″ dimension is vertical and the 7″ dimension is horizontal. Using a soft lead pencil, draw a horizontal line across the sheet 1 inch from the bottom edge. Then mark off on the line three 1-½″ segments about ½″ apart. Label the 1-½″ segments as follows:

First sheet
1. Citrus Red No. 2
2. "Color Added" oranges
3. Oil Red XO

Second sheet
1. Citrus Red No. 2
2. Oranges not stamped "Color Added"
3. Oil Red XO

Make a solution of mineral oil in ethyl ether by dissolving 5 grams of the oil in 95 ml. of the ether. Stir until well mixed. Transfer to a 100 ml. graduated cylinder. Immerse one rolled sheet of the paper in the mineral oil solution for a few minutes. Remove and dry it by suspending it in air. Treat the second sheet in the same way. (Note—Ether is highly inflammable and should be kept away from flames or electric heating elements.)

Make a mixture of 130 ml. of acetone and 70 ml. of distilled water. Stir until mixed and store until ready for use in a glass-stoppered bottle.

Place a small piece of cotton in the bottom of a 125 ml. filtering funnel supported on a stand. Position three glass rods in the funnel in such a manner that they will support an orange so it will not touch the sides of the funnel.

Prepare standard solutions of the two dyes as follows: dissolve 10 mg. of Citrus Red No. 2 in 50 ml. of chloroform. Store in a glass-stoppered, labeled bottle, away from light.

Dissolve 10 mg. of Oil Red XO in 50 ml. of chloroform. Store in a glass-stoppered, labeled bottle, away from light.

Procedure

1) Set water to boil if steam bath is not available (for step 4).

2) Place a 125 ml. Erlenmeyer flask beneath the funnel and support an orange on the glass rods. Wash the color off a "Color Added" orange by spraying it with 25 ml. of chloroform in the form of a fine stream from a pipet. (Note: Use rubber tube on end of pipet. Avoid getting chloroform in your mouth.) Surface oils, waxes, and natural pigments, as well as the artificial color will be washed off.

3) Repeat step 2 with two more of the "Color Added" oranges, combining the washings in the same flask.

4) Transfer a portion of the solution in the flask to a 100 ml. beaker. Allow the solution in the beaker to evaporate by placing the beaker on a steam bath in the hood, or over a suitable container of hot water, if a steam bath is not available. When the solvent has evaporated, add another small portion of the solution. Continue the evaporation in this manner until all of the solution has been transferred and all of the solvent has evaporated.

5) Using another 125 ml. flask and a clean funnel, glass rods, pipet, and beaker, repeat steps 2, 3, and 4 above, using oranges not stamped as artificially colored.
While waiting for the chloroform in the two beakers to evaporate, proceed with step 6.

6) Pour the prepared solution of acetone and water into the bottom of the chromatographic tank. Transfer a 50 microliter portion (need not be measured exactly) of each of the prepared solutions of Citrus Red No. 2 and Oil Red XO to the marked sheets of filter paper prepared as indicated above, by dipping a pointed capillary tube or melting point tube into the solutions and drawing the liquid in a band along the appropriate 1-½" lines on the filter papers.
Permit the chloroform to evaporate from the paper for about 5 seconds, and retrace the line with the tip of the pipet twice more. Permit drying between applications. Finally, suspend the papers in air to dry.

7) Allow the beakers to cool after removing them from the steam bath. Dissolve the residue in each beaker in 3 ml. of chloroform. Transfer a 50 microliter portion of the "Color Added" extract to the first sheet of prepared filter paper in the same manner as you did with the authentic dye solutions; repeat on the second sheet with the extract from the presumably uncolored oranges.

8) Lower the two sheets of filter paper in the chromatographic tank so that the 9" dimension is vertical and the 7" dimension is horizontal, and suspend from a rod in such a way that the bottom edge of the paper (with the 1-½" marked segments) is immersed about ¼" in the solvent. Do not allow the papers to touch each other or the sides of the tank. Cover the tank and allow the papers to remain undisturbed for about 1 hour.

9) Remove the papers from the tank and suspend in air to dry. For each sheet of paper, measure the distance the colors have traveled. By comparing the distance traveled by the known color solutions with that of the solutions washed from the oranges, you can determine which one of these colors was used on the "Color Added" oranges, and whether either of them was used on the other oranges without the required declaration. Usually Citrus Red No. 2 will travel a distance of about 2-¼" from the point of origin, and Oil Red XO will travel about ¾" from the origin. The natural coloring materials present will remain at the origin.

For Discussion

1) Was the permitted color used on the "Color Added" oranges? Did you find any color on the unlabeled oranges? What action should be taken by the Food and Drug Administration to protect consumers if violations are found? If you found a spot that traveled a distance different from either known color, what would this indicate?

2) Could you suggest a way to determine whether added color penetrates into the flesh of the orange?

3) If the color used has been proved safe, and the orange is stamped to show its presence, is there any possible objection to use of the color? Explain.

4) How does the coloring material travel up the chromatographic paper? What determines the rate at which it travels?

5) Could a water-soluble color be used to color oranges? Why?

6) Why are certain colors called "coal tar colors"?

7) The coal tar colors used on foods were subjected to Government testing for purity as early as 1907, long before any other types of food ingredients. Why was this precaution considered necessary?

8) A new color law was enacted by Congress in 1960 as the Color Additives Amendment to the Federal Food, Drug, and Cosmetic Act. Look in your encyclopedia for information about these Amendments. Learn three ways in which this law differed from the law before it was amended.

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Jacobs, Morris. *Chemical Analysis of Foods and Food Products*, 3rd Edition. Princeton: D. Van Nostrand, 1958, 128-29.

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Mitchell, Lloyd C. "Ascending Paper Chromatography: A Way to Do It." *Journal of the Association of Official Agricultural Chemists*, XL (1957), 999-1029.
Separate paperbound copies may be obtained from the Association of Official Analytical Chemists, Box 540, Benjamin Franklin Station, Washington, D.C. 20044, at a cost of $1 each, or $.75 each when ordered in lots of four or more. Orders accompanied by payment will be sent postpaid; otherwise, postage and handling charges will be billed at cost.

"Separation and Identification of Food Colors Permitted by the Colouring Matters in Food Regulations 1957," The Association of Public Analysts, London, 1960.

FDA

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Analysis for spoilage indicators in butter

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Adapted from *Official Methods of Analysis of the Association of Official Agricultural Chemists,* 9th Edition, 1962, 206-209.

Level Senior high school chemistry

Techniques Titration
Preparation and use of a standard solution

Time required Forty-five minutes with practice or experience

SPECIAL CAUTIONS Metallic sodium can be dangerous to handle. It must be kept from contact with moisture and must be handled only with forceps. Sodium should be stored and cut to size under a layer of kerosene. In making sodium ethylate, it is best to use *absolute* ethanol* as a safety measure (see page 5).

All steps requiring ether should be performed in a hood; if no hood is available, this experiment *should not be attempted.*

**See page 7 for information on obtaining tax-free ethanol.*

ANALYSIS FOR SPOILAGE INDICATORS IN BUTTER

presented by the
Educational Services Staff
Food and Drug Administration

The Federal Food, Drug, and Cosmetic Act (FD&C Act) requires butter to contain not less than 80 percent by weight of milk fat and to be made from clean, unspoiled cream.

It is relatively easy to devise tests for fat content of foods, and it is easy to detect extraneous material by microscopic techniques. But laboratory tests to detect spoilage have presented a challenge both to the FDA chemists who enforce the law, and to industry chemists who are equally anxious to make sure that no unfit cream is accepted for buttermaking.

What happens when cream spoils or decomposes? Sweet cream first becomes "sour," and sour cream is regarded as a delicacy. Butter may be made either from sweet cream or sour cream.

The "souring" of cream is brought about by acid-forming bacteria naturally present in the milk. First, only lactic acid is produced by bacterial action on the lactose present. But as these bacteria grow and multiply, and as the souring action proceeds further, other types of bacterial action also occur. The fats are broken down into fatty acids

and glycerin. Other organisms act upon the milk protein, causing a type of "putrefaction." The cream develops offensive odors and flavors, and it is then "decomposed" within the meaning of the law. These offensive odors and flavors are described by experts as "fruity," "cheesy," "putrid," etc.

Every buttermaker has an expert cream taster who tastes every can of cream as it is delivered to make sure it is not spoiled. Food and Drug inspectors are also expert cream tasters, and they make inspections of butter plants to see that unfit cream is being rejected.

But how can the FDA chemist, or the chemist for a wholesale grocer, examine the butter to check up on whether the cream taster knew his business and did his job well?

Laboratory tests on thousands of samples of deliberately spoiled cream have shown that, as spoilage progresses, the fats are broken down into their constituent acids. The relatively simple, short chain acids—butyric, caproic, caprylic, and capric—are soluble in water. When cream is churned into butter, these soluble acids go out with the buttermilk or whey. But the complex, long-chain acids are water-insoluble. They remain in the butter.

These facts, therefore, suggest a way to show whether butter was made from spoiled cream: determine how much water-insoluble acids it contains.

As we have seen, spoilage is the result of a continuous process. There are *some* water-insoluble acids in sweet cream, more in sour cream, still more in spoiled cream.

What amount of water-insoluble acid (WIA) in butter would prove conclusively that the butter had been made from decomposed cream?

Thousands of experiments have been run in which every can of cream used in a churn of butter was examined beforehand, and the butter checked for WIA content. These experiments have shown that butter made from acceptable cream will not contain more than 400 milligrams of water-insoluble acids per 100 grams of butterfat.

This gives the chemist a tool he can use to determine whether butter meets one of the requirements of the law.

For law enforcement purposes, the determination of WIA must be very precise. A gravimetric method is used, which is long and tedious.

An FDA chemist has devised a rapid procedure that can be used as a preliminary screening test, to be followed by the official procedure when the preliminary results are suspiciously high.

The following experiment is based on FDA's rapid test for WIA.

Experiment

Problem

To determine whether butter was made from decomposed cream.

Equipment needed:

forceps
evaporating dish
glass-stoppered bottle
triple beam balance
2 Erlenmeyer flasks, 125 ml. (one with a standard ground glass taperneck)
Bunsen burner
Centigrade thermometer
2″x2″ piece of aluminum window screening
separatory funnel, 125 ml.
2 burettes, 50 ml.
burette stand
2 graduated cylinders, 100 ml. and 1000 ml.

Reagents and other supplies:

distilled water (wherever water is required except for the ice and salt bath)
kerosene, 100 ml.
absolute ethanol, 800 ml.
metallic sodium
conc. hydrochloric acid, 10 ml.
sodium carbonate, reagent grade
methyl orange (dissolve 0.1 gram in 100 ml. water)
butter, 10 grams
ice water, 300 ml.
ice and salt bath
ether
phenolphthalein indicator solution (dissolve 1 gram phenolphthalein in 100 ml. alcohol)
0.1 N hydrochloric acid, 100 ml.

TO MAKE SODIUM ETHYLATE

CAUTION: Always handle sodium with forceps under kerosene as directed.

First, place enough kerosene to cover sodium used in an evaporating dish. Take the tare weight. Using forceps, remove a portion of sodium from the laboratory supply bottle and place it in the dish. Weigh out about 1.2 grams. Still using forceps, return any remaining sodium to the original container. With the forceps, remove the 1.2-gram piece of sodium from the kerosene, dry on filter paper, and put it into 800 ml. of absolute ethanol in a 2 liter Erlenmeyer flask (CAUTION, stand back). When sodium has *completely dissolved,* store the solution in a glass-stoppered bottle. This is the sodium ethylate which you will use in step 6 of the experiment. The solution must be standardized against the 0.1 N hydrochloric acid just before use.

SUBSTITUTION OF SODIUM METHYLATE FOR SODIUM ETHYLATE

CAUTION: Blindness or death might result from internal use of methyl alcohol, and all containers should be properly labeled according to the provisions of the Federal Hazardous Substances Act. Students should be warned of these hazards—"Vapor Harmful," "May be fatal or cause blindness if swallowed," and "Cannot be made nonpoisonous."

Sodium methylate may be prepared from metallic sodium and methyl alcohol in the same way that sodium ethylate is prepared. Or, dry sodium methylate, which is less hazardous than sodium, may be purchased from a chemical supply house for about $2.25 per 4 oz. Dissolve 2.7 grams of anhydrous sodium methylate in 1 liter of methyl alcohol and standardize against the 0.1 N hydrochloric acid just before use.

TO STANDARDIZE REAGENTS

0.1 N hydrochloric acid

Dilute 8.9 ml. of conc. hydrochloric acid to 1 liter with distilled water.

Weigh approximately 0.2 gram of anhydrous sodium carbonate to the nearest 0.1 milligram. Transfer the sodium carbonate to a 300 ml. Erlenmeyer flask and dissolve in 40 ml. of water. Add 3 drops of methyl orange (0.1% in water) and titrate with the hydrochloric acid solution until the color begins to deviate from the tint of a reference solution (80 ml. of boiled and cooled water containing 3 drops of methyl orange). Boil the solution gently for 2 minutes, then cool. Titrate until color is barely different from tint of reference solution.

$$\text{Normality} = \frac{\text{g. } Na_2CO_3 \text{ x } 1000}{\text{ml. acid x } 52.997}$$

0.05 N sodium ethylate

Pipet 20 ml. standardized 0.1 N hydrochloric acid into 125 ml. Erlenmeyer flask, add 2 drops phenolphthalein indicator and titrate from 50 ml. burette with the 0.05 N sodium ethylate to pink end point.

$$\text{Normality} = \frac{20 \text{ x normality HCl solution}}{\text{ml. sodium ethylate solution}}$$

Procedure

1) Weigh out 10 grams of butter and place it in a 125 ml. Erlenmeyer flask. Heat the flask gently until the butter melts, then cool until the butter is thick and creamy.

2) Add 50 ml. of ice water to the butter in the flask and shake for 5 seconds. Insert a thermometer in the flask and immerse the flask in an ice and salt bath until the thermometer drops to 10°C. Remove the thermometer and shake the flask for 5 seconds.

3) Hold the screen over the neck of the flask. Pour off and discard the liquid layer through the screen. Add 50 ml. of ice water through the screen to the butter in the flask and shake for 5 seconds. Pour off the water. Repeat this step wth three more portions of ice water, each 50 ml.

4) Working in the hood, add 25 ml. of ether to the butter in the flask and swirl the flask until the butter dissolves. Transfer the solution to the separatory funnel. Wash the flask with a few milliliters of ether and add the washings to the funnel. Let the funnel stand for a few minutes, and then drain off the lower aqueous layer and discard it.

5) Transfer the remaining ether-fat solution from the funnel to a 125 ml. Erlenmeyer flask. Wash the funnel with a few milliliters of ether and transfer the washings to the flask.

6) Add 2 drops of phenolphthalein to the solution in the flask. Titrate the mixture with the previously prepared sodium ethylate solution until the first permanent pink color appears. Record the volume of sodium ethylate solution used.

7) Calculate the number of milligrams of water-soluble acids in your sample if 1 ml. of 0.05 N sodium ethylate solution equals 13.5 mg. of water-insoluble acids. Express your answer in terms of number of milligrams present in 100 grams of butter.

For Discussion

1) Can you suggest other possible indices of decomposition in butter? Would a determination of individual acids that may be formed—e.g., butyric acid—work?

2) Since spoilage of cream is primarily the result of bacterial action, what does this suggest regarding ways to prevent spoilage?

3) How could this WIA procedure be adapted for application to cream?

4) If FDA found that a shipment of butter had been made from decomposed cream, what action could it take to protect consumers?

5) Why cannot the consumer determine for himself whether butter is made from spoiled cream?

Bibliography

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Meyer, Lillian. *Food Chemistry*. New York: Reinhold Publishing Company, 1960, 32 ff.

Teachers have found it difficult and expensive to obtain absolute ethanol. Public schools and colleges may obtain a permit, without charge, which allows them to purchase tax-free ethanol. The necessary forms for obtaining the permit are available at the regional offices of the Internal Revenue Service, Alcohol and Tobacco Tax Unit. Inquiries may be addressed to the Assistant Regional Commissioner. If you do not know the address of your nearest regional office, you may write to the Alcohol and Tobacco Tax Division, Internal Revenue Service, Washington, D.C. 20224.

FDA

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Rapid identity of margarine and butter

FDA's SCIENCE PROJECT SERIES

Food and Drug Administration/U.S. Department of Health, Education, and Welfare

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Adapted from *Official Methods of Analysis of the Association of Official Agricultural Chemists*, 9th edition, pages 205-206

Level Senior high school chemistry

Technique Measurement of temperature at which a fat dissolves completely in a specified solvent

Time required Allow two 45-minute periods, one for preparing reagents and oil samples (oil to be held 2-3 hours in oven), and one for performing actual tests (each test requires less than 10 minutes).

SPECIAL CAUTIONS Prepare alcohol reagent under hood.

Exercise care in use of flame near alcohol reagent, the solvent used with each oil sample.

RAPID IDENTITY OF MARGARINE AND BUTTER

presented by the
Educational Services Staff
Food and Drug Administration

The consumer market has two well-defined basic food products which closely resemble one another, butter and margarine. The Federal Food, Drug, and Cosmetic Act requires that the products be correctly identified so that consumers can exercise a free choice. "Butter-leggers" have occasionally been caught mixing margarine with butter. The law does not permit mixing of the two products under any labeling.

Butter has been defined in the Butter Act of March 4, 1923, as the "food product usually known as butter, and which is made exclusively from milk or cream, or both, with or without common salt, and with or without additional coloring matter, and containing not less than 80 per centum by weight of milk fat."

A Definition and Standard of Identity has been established for margarine by regulation under the Federal Food, Drug, and Cosmetic Act (FD&C Act). The standard specifies that the product is to be made from rendered fat or oil of cattle, sheep, swine, or goats, or from any vegetable food fat or oil, or mixture of these. These fats and oils may be hydrogenated, if desired, are pasteurized, and then subjected to the action of harmless bacterial starters. The fat ingredient, or ingredients, must be intimately mixed with one or more of these dairy products: cream, milk, or non-fat dry milk.

To preserve the identity of the product all the way to the consumer, the owner of a public eating place serving margarine must: (1) display prominent signs which declare "Oleomargarine Served Here" or "Margarine Served Here," or state clearly on the menu that margarine is served, and (2) serve the margarine in triangular patties, or label each serving as margarine.

To determine compliance with the law as it relates to public eating places, and to uncover violations of the law as to the identity of the products being sold as butter, it is necessary to have an analytical test which is rapid, easy to manipulate, and readily interpreted. With such a test, a Food and Drug inspector can inspect public eating places and determine rather quickly whether margarine is being served, and, if so, whether or not the consumer is being properly informed.

This test, used by FDA inspectors, is based upon the variation in solubility of different fats in the same solvent. The exact temperature at which a fat completely dissolves in a specified solvent will vary from one fat to the next. Therefore, one fat can be distinguished from another by measuring this temperature of dissolution in the specified solvent and comparing the results with standard tables.

For brevity, we call this temperature the "critical temperature of dissolution," or CTD, and the test itself is called the CTD test.

Experiment

Problem

To determine whether a product is butter or margarine

Equipment needed

(1) To prepare alcohol reagent:

2 pipets (5 ml. size)
1 glass-stoppered bottle
1 hydrometer
distillation apparatus

(2) To prepare oil from butter or margarine product:

1 evaporating dish
1 hot H_2O funnel
filter paper
oven

(3) To calibrate test tubes:

2 Pyrex test tubes 18x150 mm.—marked at 2 ml. and 4 ml. measured by adding H_2O from buret
1 buret
1 buret stand

(4) For the test:

2 Pyrex test tubes, calibrated as above
1 Centigrade thermometer, graduated from 10° to 150°
1 Bunsen burner, or other laboratory burner
pipets, glass tube about 2-3 ml. capacity drawn to fast-flowing tip
test tube rack
tongs for the test tube

Reagents and control samples

*10 ml. 95% ethyl alcohol
5 ml. iso-amyl alcohol (b. p. 128°-132° C)
butter
margarine

*See page 7 for information on obtaining tax-free ethyl alcohol.

Advance Preparation

(1) To prepare alcohol reagent. PREPARE UNDER HOOD. The preparation of this reagent is the most critical part of the CTD test. 95% ethyl alcohol contains 94.9% by volume ethyl alcohol at 15.56°C with a density of 0.816 at this same temperature. Using a hydrometer, or other suitable laboratory device for measuring specific gravity, check the ethyl alcohol for a reading of $d\frac{25}{25}$ 0.810.

Redistil iso-amyl alcohol so that its boiling point range falls between 128° and 132° C. Iso-amyl alcohol is irritating to the eyes and respiratory passages; exercise care in working with it.

Using a 5 ml. volumetric pipet, measure two volumes, 10 ml., 95% ethyl alcohol into a glass bottle. In the same way, measure one volume 5 ml. iso-amyl alcohol and add to ethyl alcohol in bottle. Stopper well, shake, and set bottle aside. Label "alcohol iso-amyl alcohol reagent."

(2) To prepare oil from butter or margarine. Place about 3 tablespoons butter or margarine in an evaporating dish. Melt product at 60°C and hold in an oven at 60° C for 2 to 3 hours, or until H_2O and curd separate completely. Filter the clear supernatant fat through dry paper in a hot H_2O funnel (or in oven) at about 60° C. If filtered liquid fat is not perfectly clear, refilter. *Oil must be clear.*

CTD Test

Using a pipet, transfer oil to a calibrated test tube, filling tube to the 2 ml. mark. Immediately add enough prepared alcohol reagent from the stoppered flask to fill test tube to the 4 ml. mark (or add 2 ml. alcohol reagent to the test tube using a pipet).

Using the thermometer as a stirring rod, mix the oil and alcohol layers and heat gently in the flame of the Bunsen burner. Keep stirring and heating until mixture becomes clear and homogeneous. *Do not boil.* Remove the clear solution from the source of heat and keep stirring until a definite turbidity appears in the mixture. Record the temperature at the first discernible turbidity. This temperature reading is the critical temperature of dissolution, CTD. (As the test tube mixture cools, opalescence will immediately follow throughout the entire mixture.)

Interpretation of Results

The CTD's for butter oil will approach a reading of 50°C and the CTD's for margarine oil will approach a reading of 75°C. The difference in temperatures found in the CTD test for these two oils will be about 25°.

An extensive survey made by the Food and Drug Administration in 1949 before its adoption of this method as an official part of its enforcement program, found that the overall CTD range for all butters was 42°C to 53°C. The individual CTD's obtained showed no correlation with the locality of production of the butter, or with the quality of the butter.

The CTD's for margarine ran between 66°C and 75°C in this testing program. There was no correlation between the individual CTD's for margarine and the types of oils or fats from which the margarine was made.

According to this study, your test results should be sufficiently consistent, and the CTD difference between butter and margarine should be in the range of 22 to 26 degrees.

(See Felman and Lepper, Journal of the Association of Official Agricultural Chemists, 33 (1950), 492–498.

For Discussion

1) Study the labels of a package of butter and a package of margarine.
What differences do you notice in declaration of content?
2) Would substitution of margarine for butter be considered a danger to health? An economic fraud? Why? Discuss other types of substitution or "adulteration" in the same category.
3) What are some of the other requirements of the law for the manufacturers of products such as margarine or butter?
4) Describe the process by which the FDA sets a Definition and Standard of Identity for a product such as margarine.
5) What action can the Food and Drug Administration take to protect consumers if it finds that a package label does not honestly identify the product?

Bibliography

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"Know Your Butter Grades," Marketing Bulletin No. 12, U. S. Department of Agriculture. Free on request from USDA Office of Information, Washington, D.C. 20250.

"Oleomargarine, Margarine: Definition and Standard of Identity," Part 45, F.D.C. Regs. Free from Consumer Inquiries Staff, Food and Drug Administration, Washington, D.C. 20204.

Additional references to be found in a public or technical library: (a) *Code of Federal Regulations,* Title 21, Chapter I, Part 3—Section 3.17 "Labeling of Oleomargarine or Margarine." (b) "Composition of Certain Margarines," statement of Council on Foods and Nutrition, American Medical Association, *Journal of American Medical Association,* 179 (March 3, 1962), 719. (c) Felman, Harold A., and Lepper, Henry A. "The Critical Temperature of Dissolution as a Rapid Test to Distinguish Oleomargarine from Butter," *Journal of the Association of Official Agricultural Chemists,* 33 (1950), 492. (d) Mehlenbacher, V. C. *The Analysis of Fats and Oils.* Champaign, Ill.: Garrard Press, 1960, 236.

Public schools and colleges may obtain a permit, without charge, which allows them to purchase tax-free ethanol. The necessary forms for obtaining the permit are available at the regional offices of the Internal Revenue Service, Alcohol and Tobacco Tax Unit. Inquiries may be addressed to the Assistant Regional Commissioner. If you do not know the address of your nearest regional office, you may write the Alcohol and Tobacco Tax Division, Internal Revenue Service, Washington, D.C. 20224.

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Identity of synthetic colors in foods

FDA's SCIENCE PROJECT SERIES

U.S. Department of Health, Education, and Welfare
Public Health Service
Consumer Protection and Environmental Health Service
Food and Drug Administration

U.S. DEPARTMENT OF HEALTH, EDUCATION & WELFARE
OFFICE OF EDUCATION

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Adapted from *Journal of the Association of Official Agricultural Chemists,* 38 (1955), 796; 45 (1962), 767.

Level Senior high school chemistry

Technique Paper chromatography

Time required Three 1-hour lab periods

The chromatography step requires from 4 to 6 hours for full development Students could begin the experiment at the start of the day and let the process proceed until after school. This step could also be begun, the paper removed and dried, and put into the developer the next day for further development. (The schedule may be adapted to fit the situation.)

SPECIAL CAUTIONS The developing solution used for the chromatography should be prepared and used in the hood or in a well-ventilated room to prevent any possible danger from inhalation or fire.

IDENTITY OF SYNTHETIC COLORS IN FOODS

presented by the
Educational Services Staff
Food and Drug Administration

Artificial colors make food more attractive and hence contribute to the pleasures of eating. The Federal Food, Drug, and Cosmetic Act (FD&C Act) permits the use of safe artificial colors, except where this would result in consumer deception. Artificial color is prohibited if it would make the product appear better than it really is. The presence of artificial color must be declared on the label of a food. You have undoubtedly seen oranges with the words "Artificially Colored" stamped right on them.

The law also requires that food colors be proved safe before use. But this was not always so. Sometimes imported candies of 100 years ago were colored with poisonous mineral pigments of lead, arsenic, copper, and chromium. Then in 1858 the first organic dye, an aniline dye, was synthesized by William Henry Perkin. This was the beginning of the "coal-tar color" industry, so-called because aniline was made by distillation of coal. These synthetic colors soon replaced the poisonous mineral pigments.

However, the various illnesses suffered by workers in the dye industry in Germany caused real concern about the safety of these new colors for food use. The harmful effects of the coal-tar colors were believed largely due to impurities present, particularly arsenic and lead.

As early as 1907, only 1 year after the first Federal Food and Drugs Act was passed, food and color manufacturers asked the Government to list the coal-tar colors that could be safely used in foods, and to set up a system of testing or "certification" of every batch of permitted color. The tests were designed to determine whether the color was free of harmful impurities. Seven colors were listed at that time as safe for use in foods. Foods containing unlisted colors, or colors from uncertified batches, were regarded as "adulterated" and could not be sold.

The voluntary system of color certification was written into the law as compulsory in 1938. The "coal-tar" colors were getting more Government attention at that time than any other class of food ingredients.

Then, in the 1950's, something happened to bring the safety of food colors into question again. There were two separate outbreaks of poisoning of children from eating highly-colored Halloween candy and popcorn. One of the colors was the one then used to color oranges. The other was an orange color used in many different foods.

In each case the amount of color used in the product was far in excess of that normally used. But, the fact that *any* amount of these tested colors could cause illness was startling.

This discovery set in motion a chain of events that led to a still stronger color law in 1960 called the "Color Additive Amendments" to the Federal Food, Drug, and Cosmetic Act. This law provided that all of the permitted food, drug, and cosmetic colors be retested for safety, using more modern scientific techniques—techniques which had not previously been available to the scientific community. The new amendments also gave FDA the authority to limit the amount of color that may be used. The law applies to *all* food, drug, and cosmetic colors—not just coal-tar colors.

The Food and Drug chemist must know how to separate artificial colors from foods, and how to identify them. There are hundreds of coal-tar colors and other colors that are *not* permitted in foods.

Experiment

Problem

To detect and to determine the probable identity of synthetic color additives in foods

Background Information

This procedure is adapted from procedures in two publications:

Dolinsky, M., and Stein, C. "Application of a Liquid Anion Exchange Resin to the Separation of FD&C Colors from Foods," *Journal of the Association of Official Agricultural Chemists,* 45 (1962), 767.

Sclar, R. S., and Freeman, K. A. "Chromatographic Procedures for the Separation of Water-Soluble Acid Dye Mixtures," *Journal of the Association of Official Agricultural Chemists,* 38 (1955), 796.

Amberlite LA-2 (manufactured by Rohm and Haas, Independence Mall West, Philadelphia, Pa. 19105), is a high molecular weight organic amine. In acid solution, this resin forms salts with the food colors which are soluble in organic solvents. This provides a convenient way to separate the colors from food constituents. It is the basis of the procedure described below for the preparation and extraction of the sample. The probable identity of the colors may then be determined by paper chromatography.

Reagents:

normal butyl alcohol
hexane
saturated solution of ammonium sulfate
litmus paper, blue
1 percent solution of acetic acid
10 percent solution of LA-2 Amberlite Resin in butyl alcohol
1 percent solution of sodium chloride
0.1 percent solutions of FD&C certified colors
10 percent solution of reagent ammonium hydroxide in water

Apparatus:

small separatory funnels
Whatman No. 1 filter paper sheets for chromatography
small beakers
melting point tubes
American Medical Museum Jar No. 11, or any jar suitable for ascending chromatography, such as a small, square aquarium.

Procedure

1. Preparation and extraction of sample (a, b, c, or d).

a. Select an artificially-colored beverage, such as grape soda. Pour approximately 25 ml. into the separatory funnel. Add 10 ml. of the resin solution. Shake the separatory funnel gently until the color transfers to the upper (resin in butyl alcohol) layer. A drop to ½ ml. of saturated ammonium sulfate solution should separate any emulsion formed

by a too vigorous shaking. Draw off and discard the lower layer.

b. Dissolve 2 grams of a strongly-colored gelatin dessert in 20 ml. of hot water. After cooling, transfer the solution to a separatory funnel and extract as in a.

c. Dissolve 2 grams of a strongly-colored hard candy in 20 ml., or less, of 1 percent acetic acid and extract as in a.

d. Remove the coating of colored candy-coated chewing gum (two pieces, approximately 3 grams) by soaking it for a short time in two 5 ml. portions of 1 percent acetic acid. Extract the color as in a.

2. Washing of extract.

Shake the resin layer with one to three 10 ml. portions of water until the lower layer is not acid to blue litmus paper. Discard the washes. Mix 20 ml. of hexane with the resin layer and discard any water layer that separates.

3. Recovery of colors.

Extract the color from the hexane-butyl alcohol resin layer by shaking gently with 1 to 2 ml. portions of 10 percent ammonium hydroxide. No more than three shakeouts should be required to remove all of the color. Collect the color solution in a small beaker. If FD&C Blue No. 1 or FD&C Violet No. 1 is thought to be present, add acetic acid by drops to the aqueous extract until the solution is neutral to litmus.

4. Separation and identification of certain food colors by paper chromatography.

(a) Chromatograms with known colors:

The diagram shown on page 7 is that of a typical paper chromatogram of FD&C Blue No. 1, FD&C Green No. 3, FD&C Violet No. 1, FD&C Red No. 2, FD&C Red No. 3, FD&C Red No. 4, FD&C Yellow No. 5, and FD&C Yellow No. 6.

Use melting point tubes open at both ends, or a pointed glass rod, for spotting the papers. Spot the solutions of known colors about 1 inch from the edge of a sheet of paper cut to fit the chromatograph jar. Allow the spots to dry. Add 1 percent salt solution to the jar to a depth of ¼ to ½ inch. Suspend the paper in the jar so that the lower edge touches the liquid, but so that the liquid is below the spots. Cover the jar. Allow the chromatogram to develop until the solvent has moved approximately 8 inches (about 1 ½ hours). Remove and dry the paper.

(b) Chromatograms of the unknown color solution:

Spot the unknown color which has been extracted from the food about 1 inch from the edge of a paper. If it is a mixture of color of unequal concentrations, it may be easier to observe if spots of different concentrations are separated by chromatography. The dilute solutions will make a more concentrated spot by multiple spotting. To keep the spot small, allow each spotting to dry before adding the next drop of color to the same area.

Chromatograph the mixture of unknown colors as in a. Decide on the probable identity of the colors by the position and color of each colored zone in the finished chromatogram.

Both chromatograms may be developed simultaneously in the same jar.

To obtain FD&C certified colors for making chromatograms with known colors, write to the following manufacturers:

General Sales Manager
H. Kohnstamm and Co., Inc.
161 Avenue of the Americas
New York, N. Y. 10013

Product Manager—Certified Colors and Food Acids
National Aniline Division
Allied Chemical Corporation
40 Rector Street
New York, N. Y. 10006

Line of the solvent front

F D & C Blue No. 1

F D & C Green No. 3

F D & C Violet No. 1

F D & C Red No. 2

F D & C Red No. 3

F D & C Red No. 4

F D & C Yellow No. 5

F D & C Yellow No. 6

Line of original spots

For Discussion

1) How do the Government safety clearance requirements for food colors differ from the safety requirements for other food additives?

2) How does a color manufacturer prove to the Government that a color is safe for use in foods?

) Examine the labels of some food colors in your kitchen. What label statement do you find there that is of special interest? Is a similar statement found on labels of other foods containing artificial color?

4) Why do some colors move up the filter paper "wick" faster than others? Explain how this fact is used to identify substances by paper chromatography. Could the rate of migration be likened to a chemical "fingerprint"? Why?

5) Were the colors you found in the food or beverage you analyzed *permitted* colors? What action could the Government take if nonpermitted colors were found?

6) Can you think of ways in which artificial colors might be used to deceive the consumer as to the quality of the food? How might you check a food quickly to see if it contained a water-soluble color not declared on the label?

7) Does your State have a pure food law? If so, what are its provisions regarding artificial colors?

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Paffenbarger, and Pearlman. *Frontiers of Dental Science.* Washington, D.C.: National Science Teachers Association. Available from Scholastic Book Services, 904 Sylvan Avenue, Englewood Cliffs, N. J. 07632, for 50 cents. (See page 41, "Chromatography.")

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